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(71) Applicant: BEECHAM GROUP LIMITED, Beecham House Great West Road, Brentford, Middlesex (GB)

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(72) Inventor: Garman, Andrew John, 37 Oakdene Road, Brockham Betchworth Surrey (GB)
Inventor: Wheeler, Alan, 6 Church Terrace, South Holmwood Surrey (GB)

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(74) Representative: Dawson, Hugh Bainforde, Dr. et al,
European Patent Attorney BEECHAM
PHARMACEUTICALS Yew Tree Bottom Road, Epsom,
Surrey KT18 5XQ. (GB)

(54) Modified allergens.

(57) Allergens modified with polysarcosine, pharmaceutical compositions containing them, a process for their preparation, and their use in the treatment of allergies.

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MODIFIED ALLERGENS

This invention relates to modified allergens, to a process for their preparation, and to their use in the therapy of allergic humans.

Many people are allergic to allergenic materials such as pollens, weeds and house dust. Such allergies have been conventionally treated by the administration to the sufferer of repeated gradually increasing doses of the relevant allergen, to build up resistance to the allergen. This is known as desensitisation.

In our U K Patent No 1 282 163 is described one improved form of therapy, in which the allergenicity of a given allergen is reduced by treatment with glutaraldehyde. It is found that such material maintains its ability to stimulate the desired blocking antibody, and thus may be used in desensitisation therapy with a reduced risk of side effects.

An alternative approach to the problem is the modification of the allergen such that on administration it suppresses the production of IgE antibodies specific to the unmodified allergen. Such an approach

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is described in Dutch Patent Application No. 7 709 025
(now also published as U.K. Patent No. 1 578 348),
wherein it is stated that allergen-polyethylene glycol
conjugates are capable of eliciting the therapeutically
5 desirable effect of suppressing in particular allergen
specific IgE production. These materials are also
stated to be substantially non-allergenic and non-
immunogenic.

It should be noted that although in this Dutch
10 Patent Application it is suggested that other
polymers such as polyvinylalcohols, polyvinylpyrrolidones,
polyacrylamides and homopolymers of amino acids may be
used in place of polyethylene glycol, only polyethylene
glycols are illustrated.

15 We have now discovered that allergens modified
with polysarcosine have the ability to suppress the
production of IgE antibodies specific to unmodified allergen.

Nowhere in the Dutch Patent Application referred
to above is the use of this one specific material,
20 polysarcosine, in any way disclosed or suggested.

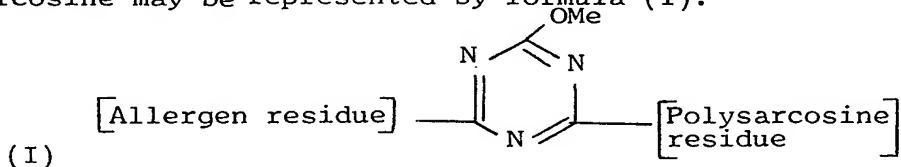
Accordingly the present invention provides an
allergen having bound thereto polysarcosine.

The allergen will be in the form of an extract
of whole allergen, as is conventional. Suitable whole
25 allergens from which the extract can be obtained include
pollens, such as grass pollens, for example rye; weeds,
such as ragweed; house dust mites; venoms, such as
bee venom. Often the whole allergen will be ragweed,
or a mixture of grasses, preferably a mixture of grasses.

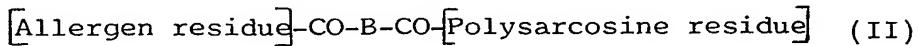
30 The techniques for binding molecules to allergens
are well known to the skilled worker, and are illustrated
for example in the Dutch Patent Application.
Basically, the binding depends on reacting active groups
35 on allergen molecules with active groups on the poly-
sarcosine, and if necessary or convenient providing
such active groups as a first step.

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While any appropriate known technique may of course be employed, we have found that 2,4-dichloro-6-methoxy-s-triazine is a most useful binding agent. In such cases, the bridges formed between allergen and polysarcosine may be represented by formula (I):



Other suitable bridges between the allergen and the polysarcosine may be represented by formula (II):



wherein B is a hydrocarbon chain of 1 to 4 carbon atoms, optionally containing a double bond. Examples of such bridges include -CO-CH₂-CO-, -CO-CH₂-CH₂-CO-, and -CO-CH=CH-CO-.

The binding group is suitably joined to an amino group on the allergen, and to a H(CH₃)N- group on the polysarcosine.

The polysarcosine for use in this invention may have any convenient molecular weight. However we have found that polysarcosine of molecular weight in the range 2000 to 12,000 is suitable, more suitably 3000 to 9000, most suitably 4000 to 8500. It has been found that a molecular weight of 7500 to 8500 is particularly useful. Polymers of molecular weight in this range are commercially available, but of course can be synthesised in conventional manner if desired or necessary. When used herein in relation to polysarcosine, molecular weights are number average molecular weights.

It will be appreciated by the skilled man that each allergen molecule will have a number of suitable sites for binding of polysarcosine molecules. It will be a routine matter for the skilled man, now we have discovered the advantages obtained thereby, to determine by simple experiment suitable binding levels for

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different allergens and different molecular weight polysarcosines to give the desired activity (IgE suppression). However, by way of illustration we have found that suitably 1 to 70% of the allergen sites are bound. More 5 suitably 4 to 40% of the sites are bound. The level of site binding is easily determined, for example as illustrated hereafter in the Examples.

From the above it will be appreciated that preferred materials of this invention include those 10 wherein the allergen is mixed grasses, the polysarcosine has a molecular weight of 7500 - 8500, the bridge between allergen and polysarcosine is formed by 2,4-dichloro-6-methoxy-s-triazine, and 4 to 40 % of the allergen sites are bound.

15 Modified allergens according to this invention may, depending on for example the allergen, polysarcosine, and degree of binding, have a significant level of retained allergenicity (as well as the necessary IgE suppression activity). Such allergens form a useful 20 class of materials within the invention. By way of example, which is not to be taken as limiting in any way, such materials may suitably have an allergenicity not less than 10% of the unmodified allergen.

In the art, materials consisting of allergens 25 having bound thereto chemicals, such as for example the materials described in the said Dutch Patent Application, are often referred to as 'conjugates'. Using this terminology, the materials of the present invention can be referred to as a conjugate of an allergen and 30 polysarcosine.

The materials of this invention suppress IgE production specific to the unmodified allergen. They may therefore be used in the therapy of allergy in humans. For example, if a patient is allergic to 30 ragweed, then a material according to this invention in which the allergen is ragweed would be used.

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The materials of the present invention may be employed as the active agents in compositions such as vaccines. Such compositions are well known to those skilled in the art and comprise a sterile liquid vehicle
5 in which the active agent is dissolved or suspended. If suspended, the particles of active agent should be small enough not to block the orifice of an injection needle. Certain additives such as tyrosine are often included in such compositions and are believed to provide a support and prolonged slow release of active
10 material in vivo.

Usually a patient receiving treatment with such a composition is administered a number of injections, spread over a period of weeks or days.

15 It is also believed that the materials of the invention may be active via other routes of administration, such as the nasal mucosa, when administration can be as a liquid spray or as a dry powder.

Accordingly in an additional aspect the invention
20 provides a pharmaceutical composition comprising a material of the invention together with a pharmaceutically acceptable carrier.

A preferred composition of this invention is as a vaccine.

25 The composition of this invention may, by way of illustration, suitably contain 1 to 10000 P.N.U. of modified material.

Normally of course doses towards the lower end of this scale will be more suitable for early in the therapy; doses towards the higher end of this scale for later
30 in the therapy.

The invention also provides a process for the preparation of the materials of this invention, which process comprises binding the polysarcosine to the allergen.

5 The binding reaction can be carried out in any suitable manner, following long established procedures.

For example, the polysarcosine may first be reacted with a compound D-X-E wherein X is a bridging group, and E is a group reactive with a group on
10 polysarcosine and D is a group reactive with a group on the allergen.

One suitable example of a compound D-X-E is 2,4-dichloro-6-methoxy-s-triazine (DCMT).

Alternative examples of D-X-E include compounds
15 wherein X is as previously defined for B, and D and E are carboxyl groups. In such cases of course E may if necessary be activated prior to reaction with the polysarcosine, and D may if necessary be protected during this reaction. Other examples of D-X-E include
20 cyclic anhydrides.

The thus formed D-X- polysarcosine may then be bound to the allergen.

This reaction is suitably carried out in common alkaline buffer systems, for example 0.1M Na₂CO₃/NaHCO₃,
25 pH 9.0, using a protein concentration of typically 10 mg/ml. Reaction is usually carried out at 30°C for 1 to 3 days. Unreacted polysarcosine may suitably be separated by gel filtration using Sephadex G75 or by other convenient methods, such as ion exchange
30 chromatography.

The degree of allergen substitution in the reaction can generally be controlled by addition of different amounts of activated polysarcosine.

The following Examples illustrate the invention.

Example 1(i) Preparation of DCMT

Cyanuric chloride (Aldrich, 22.08 g, 0.12 mole) was added to a mixture of methanol (120 ml), water (15 ml) and sodium bicarbonate (20.16 g, 0.24 mole). The reaction mixture was stirred at 30°C for 30 minutes by which time CO₂ evolution had ceased. Water (80 ml) was added and the mixture stirred for 5 minutes. The white solid was filtered off and dried over P₂O₅. The product was then twice recrystallised from cyclohexane. Yield 10.3 g. m.p. 89-90°C (lit. 88-90°C) ¹H-N.m.r. (CDCl₃): δ, 4.17 (s, 3H, OCH₃). Element analysis: N, 23.33 (23.30); C, 26.66 (26.65); H, 1.66 (1.35); Cl, 39.44 (39.86).

(ii) Preparation of DCMT-activated Polysarcosine 4800

Polysarcosine (Miles, No. average mol. wt (\bar{M}_n)=4,800, 950 mg) was dissolved in distilled water (3.3 ml) and acetone (3.3 ml) added. The pH was adjusted to 7.0. 2,4-Dichloro-6-methoxy-s-triazine (114 mg 3-fold molar excess) was added with stirring, and the pH maintained at 7.0 by addition of 0.5M NaOH from an autoburette; 0.82 ml was consumed by the reaction (theoretical = 0.79 ml). Undissolved DCMT crystals were removed by centrifugation and the supernatant applied to a column of Sephadex G25 SF (180 ml) equilibrated with distilled water. The peak eluting in the excluded volume of the column was pooled and the activated polysarcosine recovered by lyophilisation. Yield 637 mg. N; 18.10, 19.14 (19.88); C: 46.12, 45.90 (49.92); H, 6.81, 6.56 (6.86); Cl; (0.84, 0.82 (0.71). Calculated values assume M.W. 4,800 and that the product is anhydrous.

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(iii) Preparation of Rye/Poly-Sar
4800

Rye grass pollen extract (25 mg) was dissolved in 2.5 ml 0.1M $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ buffer pH 9.7 (9.5 at 37°C) containing 0.02% NaN_3 . DCMT-activated poly-Sar was added as shown in Table 1, Samples 1, 2, 3 and 4. Reaction 5 was allowed to proceed for 3 days at 37°C after which each reaction was stored frozen prior to chromatography. To each reaction mixture was added 8M KSCN in PBS (2.5 ml) and the solution was subjected to gel filtration upon a Sephadex G75 column equilibrated with 4M KSCN in PBS. To 10 the control was added 8M KSCN in PBS (2.5 ml) followed by 4M KSCN in PBS (10 ml); this solution was allowed to stand at room temperature for ca. 4 hours. The peak eluting in the excluded volume was pooled, dialysed against 30 mM NH_4HCO_3 and lyophilised.

(iv) Analysis of Allergen/Poly-Sar

Allergen primary (1°) amino groups were assessed by a standard assay using trinitrobenzene sulphonic acid; the sarcosine content of the conjugates was measured by amino acid analysis; and then the corresponding number of primary amino groups of the allergen modified with 15 polysarcosine was calculated. The degree of polysarcosine substitution was expressed as a percentage of allergen primary amino groups modified, as shown in Table 1 following.

(v) Results

The results obtained are shown in Table 1, Example 1.

Example 2

(i) Polysarcosine_X* (where X = \bar{M}_n) obtained from a commercial source was activated by reaction with DCMT as described in Example 1, (ii).

(ii) Preparation of Rye/polysarcosine_X conjugates

Rye-grass pollen extract (30 mg) was dissolved in 5 0.1M NaHCO₃/Na₂CO₃ buffer (pH 9.0, 3 ml) and DCMT-activated polysarcosine_X (proportions shown in Table 1) added. After 20 hours at 30° each reaction mixture was frozen prior to chromatography.

The pH of the mixture was brought to 7 and 8M 10 potassium thiocyanate in PBS added (3 ml). This solution was applied to a Sephadex G75 column equilibrated with 4M KSCN in PBS. Elution with 4M KSCN/PBS afforded separation of the conjugate, fractions of which were pooled, dialysed against 10 mmole NH₄HCO₃ until no more SCN[⊖] ions 15 could be detected, and lyophilised.

The buffer control was adjusted to pH 7, diluted with an equal volume of 8M KSCN/PBS and allowed to stand for 4 hours before dialysis and lyophilisation.

20 The products were analysed by the methods described in Example 1 (iv).

Sample Nos. 5-13 (Table 1)

[* Miles or Sigma]

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Example 3

(i) Preparation of Sarcosine-N-carboxyanhydride (Sar NCA)

Phosgene was bubbled through a solution of sarcosine (16 g, 0.18 mole) in redistilled dioxan (600 ml, dried prior to use over 3A molecular sieves) until saturation was achieved (approx. 1 hour). The solution was heated at 60-80° for 30 mins and then stirred at room temperature for 16 hours while nitrogen was bubbled through it. Dioxan was removed under reduced pressure and the residue triturated with diethyl ether (2 x 100 ml). Recrystallisation from chloroform gave Sar NCA 8.52 g, 41% of theoretical.

(ii) Preparation of polysarcosine₆₅₀₀

Sar NCA (1.1 g, 9.56 mmole) was dissolved in dry, redistilled pyridine (98 ml) and distilled water (20 mg) added. The solution was stirred at room temperature for 96 hours, pressure being relieved via a calcium chloride drying tube. Excess diethyl ether was added and the precipitated material filtered, washed with diethyl ether then dissolved in water and lyophilised. Yield = 0.454 g, 73% theoretical of $\bar{M}_n = 6,500$ (determined by an end-group titration).

This material was activated by reaction with DCMT as described in Example 1 (ii); the DCMT-activated polysarcosine₆₅₀₀ thus obtained was conjugated with Rye extract as described in Example 2 (ii).

Sample Nos. 14-16 (Table 1).

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Example 4

(i) Sar NCA (2 g, 22.5 mmole), prepared as described in Example 3 (i) was added to a solution of sarcosine (0.63 mg) in redistilled N,N-dimethylformamide and stirred at room temperature for 24 hours. Excess 5 diethyl ether was added and the precipitated product collected by filtration, washed with diethyl ether and dissolved in water. This solution was passed down a Sephadex G15 column using water as eluant. The fractions containing polysarcosine were pooled and 10 lyophilised to give a white solid, yield = 0.785 g, 87% theoretical of \bar{M}_n = 3400.

This material was reacted with DCMT in the manner described in Example 1 (ii) and the product conjugated with Rye-grass pollen extract as described in Example 15 2 (ii).

Sample No. 17 (Table 1).

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Example 5

(i) Preparation of N-phenylmethoxycarbonylsarcosine
(ZSarOH)

Sarcosine (8.9 g, 0.1 mole) was dissolved in 5N NaOH (100 ml) and the solution cooled to 0°C. Benzyl-chloroformate (35 g, 0.2 mole) and 5N NaOH (20 ml) were added alternately, dropwise, over twenty minutes and the reaction mixture was stirred for a further three hours at room temperature.

The basic aqueous solution was washed with diethyl ether (3 x 80 ml), acidified to pH 4 and extracted with chloroform (3 x 100 ml). The combined chloroform extracts were dried over MgSO₄ and the solvent removed under reduced pressure to leave ZSarOH as a colourless oil (20.1 g, 88%).

H-nmr: CDCl₃ + TMS, δ,
15 9.9 (s, 1H, COOH),
 7.3 (s, 5H, Ph),
 5.2 (s, 2H, PhCH₂O),
 4.1 (s, 2H, NCH₂),
 3.0 (s, 3H, NCH₃).

(ii) Preparation of Sarcosine-N-carboxyanhydride (SarNCA)

ZSarOH (4.0 g, 17.9 mmole) was dissolved in sodium-dried diethyl ether (25 ml) and phosphorus tribromide (2 g, 7.4 mmole) added over ten minutes. After stirring for sixteen hours, petroleum ether (bpt, 40-60°) (100 ml) was added and the reaction mixture cooled to 4°C for four hours to aid crystallisation. The white crystalline solid which separated was collected, washed copiously with petroleum ether, recrystallised from ethyl acetate/petroleum ether at -78°C and stored in vacuo over phosphorus pentoxide.

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Yield (1.45 g, 70%)

M.pt. 102-104°C (dec)

¹H-nmr: CDCl₃ + TMS, δ

4.1 (s, 2H, -CH₂-),

5 3.0 (s, 3H, NCH₃)

Petroleum ether and ethyl acetate were dried over molecular sieves (3A) prior to use.

(iii) Preparation of polysarcosine_X

N,N-Dimethylformamide (DMF) was distilled from benzene-1,3-dicarboxylic acid and sequentially dried 10 over three batches of 3A molecular sieves. The glassware used in the reaction was baked for 48 hours at 130° prior to use.

Freshly recrystallised sarcosine-N-carboxyanhydride (0.46 g, 4 mmole) was dissolved in the required volume 15 of N,N-dimethylformamide (see table below), the flask sealed with a rubber septum cap and the solution stirred at room temperature for 96 hours. The pressure due to carbon dioxide formation was lowered periodically via a calcium chloride drying tube attached to a syringe 20 needle. Diethyl ether (3 volumes) was added and the precipitated polymer filtered off, washed copiously with diethyl ether and air-dried. The product was dissolved in the minimum volume of water necessary and desalted on a Sephadex G-15 column using water as eluant. Fractions 25 containing the polymer were pooled and lyophilised to give the product as a white solid in greater than 75% of the theoretical yield.

Molarity of SarNCA in DMF	No. average mol. wt. of product (\bar{M}_n)				
	Run No.	1	2	3	Pooled* Sample
0.04		2350	2100	2135	2260
2.0		7580	7740	8160	7800

* Polysarcosine samples of similar \bar{M}_n were pooled, lyophilised and analysed again.

(iv) Preparation of DCMT-activated Polysarcosine_X

Polysarcosine (\bar{M}_n 2260, 900 mg) was dissolved in distilled water (3 ml) and the pH adjusted to 7.0

- 5 2,4-Dichloro-6-methoxy-s-triazine (144 mg, 2 eqs) in acetone (3 ml) was added with stirring and the pH maintained at 7.0 by the addition of 1M NaOH from an autoburette: 1.04 ml was consumed by the reaction (theoretical uptake = 0.81 ml). Unreacted DCMT was
10 removed by filtration and by subsequent diethyl ether washes (5 x 3 ml). The aqueous solution was desalted on Sephadex G-15 equilibrated with distilled water. The peak eluting at the excluded volume was pooled and lyophilised to give DCMT-activated polysarcosine₂₂₆₀ (610 mg, 64%). % Cl; 1.57 (1.48, theoretical)

15 Polysarcosine₇₈₀₀ was activated by reaction with DCMT in a similar fashion.

(v) Conjugation of DCMT-activated polysar_X materials prepared as described above was undertaken in a

- 20 similar fashion to that described in Example 2 (ii). Sample Nos. 18-24 (Table 1).

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Example 6

(i) Polysarcosine_X prepared as described in Example 5 (iii) was reacted with DCMT by the method described in Example 5 (iv) to give the DCMT-activated polysarcosine_X used below.

(ii) Preparation of Ragweed/polysarcosine_X Conjugates

5 Ragweed pollen extract (30 mg) was dissolved in 0.1M NaHCO₃/Na₂CO₃ buffer, pH 9.0 (3 ml). DCMT-activated polysarcosine_X (amounts as shown in Table 1) was added and the reaction mixture allowed to stand at 30°C for 22 hours. The reaction mixture was then stored
10 frozen prior to chromatography.

15 0.1M Tris-HCl buffer, pH 9.3, (2 ml), was added to the reaction mixture, which was then applied to a DEAE 52 ion-exchange column equilibrated with 0.1M Tris-HCl buffer, pH 9.3. Elution of the column at this pH gave two peaks. These fractions were dialysed against 10mM NH₄HCO₃ and lyophilised. Analysis revealed these products to be unconjugated polysarcosine.

20 After elution of these two peaks, the eluant was changed to 0.1M Tris HCl/NaOAc buffer, pH 4.2. Elution of the column at this pH gave one peak, this fraction being dialysed against 10mM NH₄HCO₃ and lyophilised to give the ragweed/polysarcosine_X conjugate.

25 The buffer control was subjected to the pH changes described above for times similar to those experienced by the conjugates during chromatography, then dialysed against 10 mmole NH₄HCO₃ and lyophilised.

Sample Nos. 25-31 (Table 1).

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Table 1Analytical Data on Allergen/PolySar Conjugates

Sample No	Mn of polysarcosine _X	wt. ratio of DCMT-activated pSar _X extract	% ¹⁰ NH ₂ [†] groups modified	μ mole PolySar in Product per mg allergen	Biological results see Exp. No. Table No.
Example 1	4,800	7/1	56	0.31	Exp 1, Table 2
		2/1	30	0.17	
		0.5/1	29	0.16	
		0/1	0	0	
Example 2	4,800	7/1	10	0.05	Exp 2, Table 2
		0	0	0	
		10/1	22	0.12	
		5/1	17	0.09	
Example 6	2,250	2.5/1	14	0.08	Exp 3, Table 2
		7/1	11	0.06	
		3.5/1	7	0.04	
		1/1	4	0.02	
Example 13	4,470	0/1	0	0	*Exp 4 Table 3

[[†] Calculated from 100 x $\frac{\text{mole PolySar per gm of product}}{\text{mole } ^{10}\text{NH}_2 \text{ groups expected for allergen content of product}}$]

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Table 1 cont.

Sample No	Mn of polysarcosine	wt. ratio of DCMT-activated dSar-X extract	% $\text{^15}\text{NH}_2$ [†] groups modified	μ mole PolySar in conjugate per mg allergen	Biological results see Exp. No. Table No.
Example 3	14	10/1	26	0.14	Exp 5, Table 3
	15	3.5/1	13	0.07	
	16	1/1	4	0.02	
Example 4	17	4.6/1	5	0.04	Exp 6, Table 3
Example 5	18	6/1	20	0.11	Exp 7, Table 4
	19	3/1	15	0.08	
	20	1/3	7	0.04	
	21	6/1	12	0.11	
	22	2.5/1	9	0.05	
	23	1/1	7	0.04	
24		0/1	0	0	

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Table 1 cont.

Sample No	Mn of polysarcosine X	wt. ratio of DCMT-activated pSar _X extract	% ¹⁵ NH ₂ groups modified	μ mole PolySar in conjugate per mg allergen
Example 6	25	-	0/1	0
	26	0.33/1	12	0.05
	27	0.73/1	25	0.10
	28	2.33/1	55	0.21
Example 7	29	0/1	0	0
	30	1/1	15	0.06
	31	2.1/1	28	0.11

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Suppression of the Developing IgE Response in BDI Mice

Method

Groups of 6-8 BDI mice are all immunised intra-peritoneally with 10 μ g of antigen adsorbed onto 0.25 mg-1.0 mg of aluminium hydroxide gel.

On days 3, 5 and 7 the animals are injected intravenously (IV) with various amounts, usually 100 μ g of the modified allergen under test, in 0.5 ml diluent, unmodified allergen or diluent (0.5 ml).

Serum is taken usually on days, 10, 17 and 24.
Serum from animals is bulked in groups at each bleeding time.

The antigen specific IgE antibody in each bulked serum sample is titrated by a standard passive cutaneous anaphylaxis test in two rats using a latent period of 48 hours.

Results are expressed at $-\log_4$ or $-\log_2$ from $^{1/4}$ of the last dilution giving a positive result.*

To show suppressive activity, materials when used for treatment, must reduce IgE levels in immunised animals to lower levels than in those animals immunised and treated with diluent or unmodified antigen.

[Diluent (PBS) is Bacto-haemagglutination buffer (Difco)].

* (A result of 0 indicates a level of specific IgE below the limit of detection.)

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TABLE 2

Suppression of the IgE response with Polysarcosine-modified Rye pollen extract.

Expmnt No		Treatment i.v.	Amount μg	Rye	spfic	IgE-log ₄	fm ₄
		Material Sample No/Other		Day	10	17	24
1		PBS	-		4	4	4
		Rye Pollen Extract	100		4	2	2
		Buffer Cont Rye, 4	"		3	0.5	0.5
		1	"		2.5	0	2
		2	"		1	0	0
		3	"		2	0	0
		PBS	-		5	4	5
		5	100		0	0.5	1
		5	10		0	0.5	2
		Buffer Cont Rye, 6	100		3	2	3.5
2		" " " ,6	10		3	3	3
				Day	17	24	45 60
		PBS	-		4	5	8 6
		Rye Pollen Extract	100		0	0.5	5 5
		Buffer Cont Rye, 13	"		1.5	4	6.5 5
		7	"		0	0	3 0.5
		8	"		0	0.5	4 2.5
		9	"		0	0	3.5 0.5
		10	"		0	0	0.5 0
		11	"		0	0	2 3
		12	"		0	0.5	3 4

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TABLE 3

Suppression of the IgE response with Polysarcosine-modified
Rye pollen extract.

Expmnt No		Treatment i.v. Material Sample No/Other	Amount /μg	Rye spfic IgE-log ₂ ^{fml 1/4}				
				Day 17	24	52	38	66
4		PBS	-		6	7	6	
		Rye Pollen Extract	25		2	3	3	
		" "	5		2.5	3	5	
		Buffer Cont Rye	13		2.5	2.5	2	
		" " "	13		0.5	1	2.5	
		12	25		0	1	1	
		12	5		1.5	0	1	
		7	25		0	0	0	
		7	5		0	0	0	
5		PBS	-		6	6.5	6	6 5
		Rye Pollen Extract	100		0	4	4	5 5
		14	"		0	0	0	0 0
		15	"		0	0	0	0 0
		16	"		0	0	0	0 0
6		PBS	-		6.5	5.5	7	6
		17	100		0	0	0	0
		17	10		0	2	3	0
		17	1		0	0	2	0
		Rye Pollen Extract	100		0	0	1	2.5
		" " "	10		2	3.5	3	4
		" " "	1		4.5	5.5	6	4

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TABLE 4

Suppression of the IgE response with Polysarcosine-modified
Rye pollen extract.

Exponent No		Treatment i.v.	Amount/ μg	Rye spfie IgE-log ₂ ^{fm_{1/4}}
		Material Sample No/Other		Day 17 24 31
7		PBS	-	4 4.5 4
		Rye Pollen Extract	100	0 1 3
		Buffer Cont Rye24	"	0.5 1.5 2.5
		18	"	0 0 0
		19	"	0 0 0
		20	"	0 0 0
		21	"	0 0 0
		22	"	0 0 0
		23	"	0 0 0

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Discussion of Results

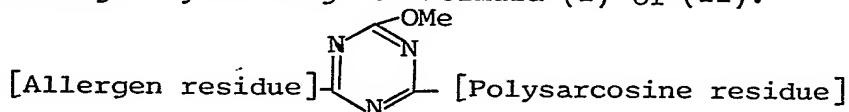
These results show that the modified materials of the invention have the desired activity.

Toxicity

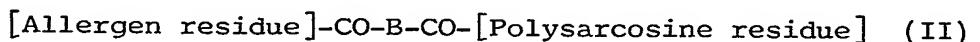
No toxic effects were observed in these tests.

What we claim is:

1. An allergen having bound thereto polysarcosine.
2. A material according to claim 1, wherein the allergen is an extract of ragweed, or of a mixture of grasses.
3. A material according to claim 1 or claim 2, wherein the polysarcosine has a molecular weight in the range of 2000 to 12000.
4. A material according to claim 3, wherein the polysarcosine has a molecular weight of 3000 to 9000.
5. A material according to any one of the claims 1 to 4, wherein the polysarcosine is bound to the allergen by a bridge of formula (I) or (II):



(I)



wherein B is a hydrocarbon chain of 1 to 4 carbon atoms, optionally containing a double bond.

6. A material according to any one of the claims 1 to 5, wherein 4 to 40% of the sites on the allergen have bound thereto the said polysarcosine.
7. A material according to claim 1, wherein the allergen is mixed grasses, the polysarcosine has a molecular weight of 7500 - 8500, the bridge between allergen and polysarcosine is formed by 2,4-dichloro-6-methoxy-s-triazine, and 4 to 40 % of the allergen sites are bound.

8. A material according to any one of the claims 1 to 7, have a retained allergenicity.
9. A pharmaceutical composition comprising a material according to any one of the claims 1 to 8, together with a pharmaceutically acceptable carrier.
10. A process for the preparation of a material according to claim 1, which process comprises binding the polysarcosine to the allergen.

11. A material according to any one of the claims 1 to 8, for use in the treatment of allergy.

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